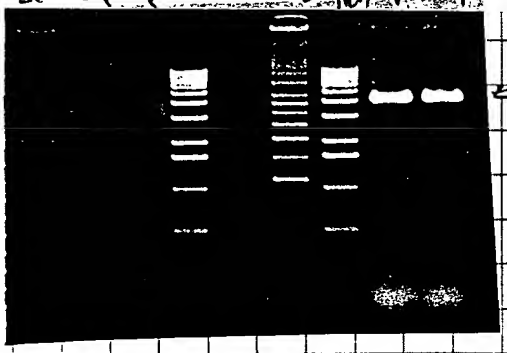
TNE

'ag N .__

12/95

Goal: To clone the TNE 35FY (mut) into ptrc99A.

PUC TNE 35FY clone #1	30	ptrc99A	5
10XR4	5	10XR2	2
H_2O	13	H_2O	11
BspHI	2	NcoI	1
	50 ul	H3	1
	37°C - 1hr.		20 ul
	Applied 5 ul to a 0.8% agarose gel. Gel ran at 180V		Applied to a 0.8% agar gel. Gel ran at 180V.



UV 7/14/95

cut out frag & freeze at -20°C.

ptrc99A/NcoI/H3 cut looks good
cut out 56 bp
4176
- 56
4120 bp

3' → 5' mut

PUCTNE 35FY #1 / BspHI gives 1Kb, 1.3Kb, + 2.7Kb frag. therefore, BspHI cuts PUCTNE 35FY #1 3X. There must be a BspHI in the insert.

13/95

EtOH ppt. Digest.
Dissolved in 20 ul TE

BspHI

5 ul
5 ul 1XB.J.
10 ul
Applied to 1 lane of 0.7% agarose gel. Gel ran at 180V

BspHI

15 ul DNA
2 ul 1XR2
2 ul H_2O
1 ul H3
20 ul
37°C - 1hr.

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ssed & Understood by me,	Dat	Invented by	Date
Lishan Xu	7/14/95		7/13/95
		Recorded by Mary Longer	

ag N .__

7/12/95

Goal: To clone the TNE 35FY (mut) into ptrc99A.

pUC TNE 35FY Clone #1	30	ptrc99A	5
10xR4	5	10xR2	2
H2O	13	H2O	11
BspH I	2	NcoI	1
	50 ul	H3	1
			20 ul

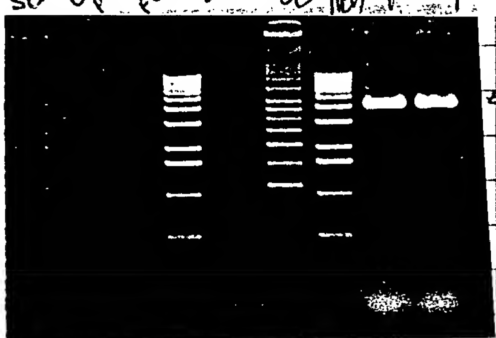
37°C - 1hr.

Applied 3 ul to a 0.9% agarose gel. Gel ran at 180V

Applied to 40.8% agarose gel. Gel ran at 180V.

SL Uncut pUCTNE35FY #1 pUCTNE35FY #1 BspHI SL 1kb ptrc99A/NcoI/H3

LX 7/14/95



← cut out frag & froze at -20°C.

ptrc99A/NcoI/H3 cut looks good
cut out 56 bp

4176
- 56
4120 bp

pUCTNE 35FY #1 / BspHI (3' stop, mut) gives 1kb, 1.3kb, + 2.7kb frag. Therefore, BspH I cuts pUCTNE35FY #1 3X. There must be a BspHI in the insert.

7/13/95

EtOH ppt. Digest.
Dissolved in 20 ul TE

BspHI ↙ ↘ BspHI

5 ul
5 ul 1XBJ
10 ul

Applied to 1 lane of 0.9% agarose gel. Gel ran at 180V

15 ul DNA
2 ul 10xR2
2 ul H2O
1 ul H3 10u/ul
20 ul
37°C - 1hr.

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ssed & Und rstood by m ,	Date	Inv nt d by / Record d by	Date
Zibhu Xu	7/14/95	May Longo	7/13/95

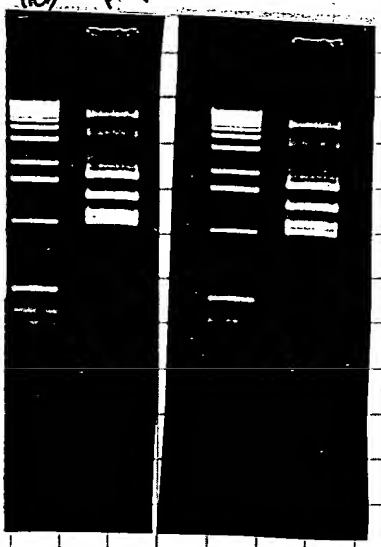
Pag N .__

4/95

pUC TNE 35FY / BSpHI → EtOH ppt. → Dissolved in 20 ul 1X buffer. 2 ul of H3 (100/ul) was added. 37°C - 1hr
7/14/95 applied to 1 lane of a 1% LMP agarose gel.
Gel run at 180V.

INV pUC TNE 35FY BSpHI/H3

1X
7/20/95



cut the 200bp frag out & freeze at -20°C.

95 Used the ~~phenol~~ 7/17/95 phenol extraction method to purify DNA.
Dissolved in 10 ul TE.

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ssed & Understood by me,	Date	Inv nted by	Date
Lizhu Yan	7/20/95	Recorded by Mary Longo	7/18/95

TNE

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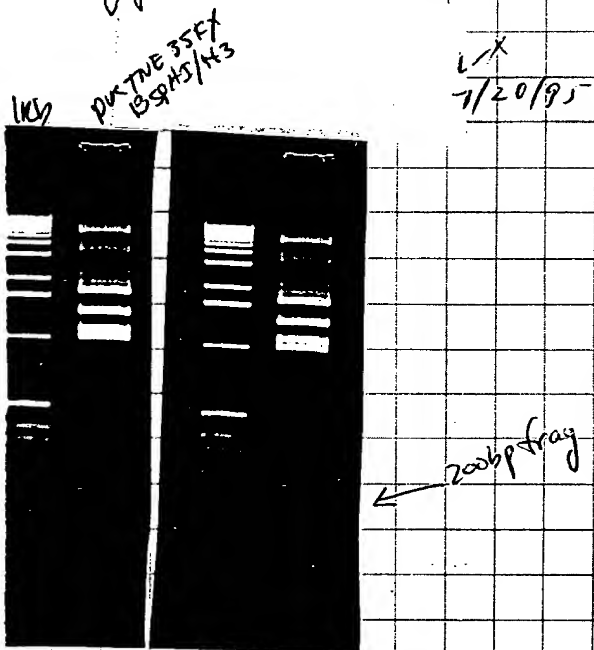
7/95

7/14/95 pUC TNE 35FY / BspHE → EtOH ppt. → Dissolved in 20ul 1X buffer. 2ul of H3 (100/ul) was added. 37°C - 1hr. applied to 1 lane of a 1% LMP agarose gel. Gel ran at 180V.



7/20/95

cut the 200bp frag out & froze at -20°C.

7/17/95

Used the ~~phenol~~ OMT phenol extraction method to purify DNA. Dissolved in 10ul TE.

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Discussed & Understood by me,	Date	Invented by	Date
Liu Yu	7/20/95	Recorded by Ming Song	7/18/95

Goal: To clone the TNE 35 FY (mut) into ptrc99A or a similar vector

New Scheme: pUCTNE 35 FY → H3 → Klenow → SphI ⇒ Sel purif 2 kb frag)
(~5.1kb)

Clone into the SmaI / SphI site of pTTQ19.

pTTQ 19	4 = ~2 ug		pUCTNE 35 FY (~50ng/ul)	20 = ~1ug
10xR4	4		10x R2	4
H_2O	30		H_2O	14
SmaI 10u/ul	2		H3 10u/ul	2
	40			40 ul

30°c - 1hr.

LX 8/3/95

Std 1 2 W) (M) 7/31/95

1 pUCTNE 35FY cut c̄ H3
2 pTTQ19 cut c̄ SmaI

cuts look good

pTTQ 19 / SmaI	40		pUCTNE 35FY/H3	40 ul
SphI 10u/ul	2		1XR2	10 ul
	42		10mM dNTP Mix	2 ul
37°c - 1hr.			Klenow	0.5 ul
				52.5

ice 5'
EDTA to 20mM
phenol extracted

Read & Understood by me, [signature] Date 8/3/95
Invented by
Recorded by [signature] Date 7/31/95

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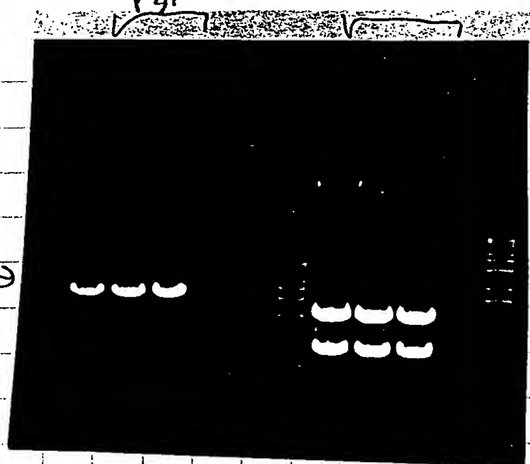
pUCTNE35FY / H3 / filled in → resuspended in 40ul 1X Rh
2ul of 10u/ul SphI
37°c — 1hr.
applied to a 0.9% agarose gel.
Gel ran at 180V.

pTTQ15 SmaI/SphI — pUCTNE35FY H3/filled in/ SphI — VX 8/3/95



JM 8/3/95

← 2kb

cut bands out + froze at -20°C.

Geneclean the frag as usual.
Dissolved in 10ul TE.
Applied 1ul to a 0.9% agarose gel.
Gel ran at 180 V.

VX 8/3/95

1kb 1 2

JM 8/1/95

1 pTTQ15 / SmaI / SphI ~ 10 ng/ul = ...
2 2kb H3/Filled in/ SphI frag ~ 20 ng/ul = .
from pUCTNE35FY

To Page N

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	8/8/95	Recorded by [signature]	8/1/95

age No.

lig

	2
TQ19 / SmaI / SphI .003 pmol/µl	1.5
cb H3 / filled in / SphI .015 pmol/µl	1
5X Ligase buffer	4
H_2O	12.5
Ligase (1U)	1
	20 µl

RT - 30 min.

Jason xformed 2 µl of the lig with 100 µl DH10B cc. std xform. Plated 10% + 90% on yet amp plates. 37°C ON

	10%	90%
#2	18	~150

picked 8 colonies into 3 mls of CG + amp100. 37°C - ON.

mp as usual. Dissolved in 50 µl TE.

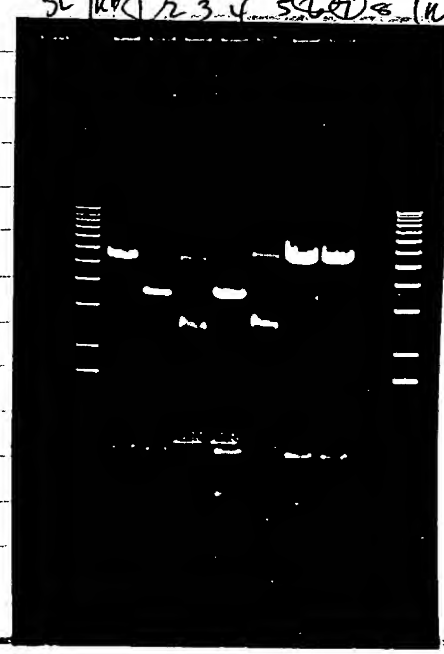
mp	3
10X RL	2
H_2O	13
SphI	1
EcoRI	1
	20

37°C - 1hr.

Applied to a 0.9% agarose gel. Gel run at 100V

4575
+ 2000
6575

2kb PUC TNE 35 PY mut into SmaI/SphI site of pTTQ19 clones cut c SphI/EcoRI



AW 8/3/95

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Disclosed & Understood by me, Lisha Xu
Date 8/3/95
Invented by
Recorded by Colleen Torpo
Date 8/3/95

TNE

Proj ct N .______
Bo k No.______

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lig

	2
TQ19 / SmaI / SphI .003 pmol/μl	1.5
kb H3 / filled in / SphI .015 pmol/μl	1
5X ligase buffer	4
H_2O	12.5
Ligase (1u)	1
	20 μl

RT - 30 min.

Jason xformed 2 μl of the lig with 100 μl DH10B cc. std xform. Plated 10% + 90% on yet amp plates. 37°C ON

	10%	90%
#2	18	~150

picked 8 colonies into 3 mls of CG + amp100. 37°C - ON

mp as usual. Dissolved in 50 μl TE.

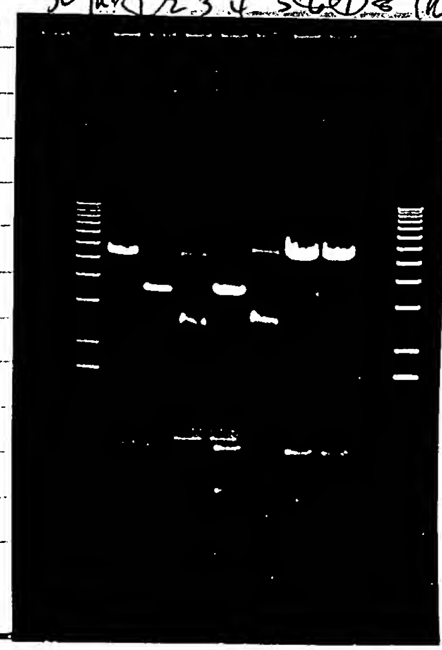
mp	3
10XRL	2
H_2O	13
SphI	1
EcoRI	1
	20

37°C - 1hr.

Applied to a 0.9% agarose gel. Gel ran at 100V

4575
+ 2000
6575

sub PUCTNE 35 PY mut into SmaI/SphI site of pTTQ19
clones cut c SphI/EcoRI
SL kb 1 2 3 4 5 6 7 8 1kb



AW 8/8/95

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Read & Understood by me,	Date	Invented by	Date
Lisha Xin	8/7/95	Recorded by [signature]	8/3/95